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(57) Abstract

New monoclonal antibodies have been found to have specific binding affinity for the CD3-T cell antigen receptor complex and not to be reactive with human monocytes. The antibodies are immunosuppressive and useful in the treatment or prophylaxis of transplant rejection or GVHD without resulting in acute reactions or EBV-associated lymphoproliferative syndrome. Representative embodiments include the BC3 monoclonal antibody and the BC3 hydridoma for producing the antibody.

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IMMUNOSUPPRESSIVE MONOCLONAL ANTIBODIES, HYBRIDOMAS AND METHODS OF TRANSPLANTATION

Field of the Invention

This invention relates to new monoclonal antibodies having specificity for the CD3-T cell antigen receptor complex, to hybridomas for producing the monoclonal antibodies, and to therapeutic methods and compositions employing the antibodies.

Background of the Invention

Recipients of allogeneic organ or tissue transplants are routinely treated with immunosuppressive agents to prevent graft rejection and, in the case of bone marrow transplantation, to prevent immunocompetent lymphoid cells from causing graft-versus-host disease (GVHD). Drugs used to control rejection or GVHD, such as corticosteroids, azathioprine, methotrexate, cyclosporine and antithymocyte globulin, have been shown to have broadly suppressive effects on cell mediated immune function. However, despite improvements in tissue matching by HLA typing and presently available improved immunosuppressive agents, acute rejection of the allograft or GVHD remain major impediments to the success of clinical transplantation. In addition, viral infections represent a significant cause of morbidity and mortality in patients immunosuppressed after transplantation. Infections with cytomegalovirus, herpes simplex virus and varicella-zoster virus result primarily from the reactivation of latent virus, although *de novo* infections have also been documented. Epstein-Barr virus-associated lymphoproliferative syndromes (EBV-LPS) have been found to occur in as high as five per cent of renal allograft recipients, and 20 per cent of cardiac allograft recipients. Latent EBV infection, together with impaired immune responses, chronic antigenic stimulation by the allograft and direct oncogenic effects of immunosuppressive drugs may represent factors that increase the incidence of EBV-related disease after organ grafting. Severe GVHD, immunosuppressive treatment, HLA incompatibility between donor and recipient and T cell depletion of the donor marrow have been

suggested as possible risk factors for the incidence of EBV-LPS in bone marrow transplant recipients.

In the search for improved immunosuppressive therapy, the use of antilymphocyte antibodies has been previously investigated. Since thymus derived lymphocytes (T cells) play a major role in the immune response to allografted tissues and marrow, treatment of transplant recipients with anti-T cell antibodies has been shown to represent a potential method of advancing immunosuppressive therapy. The administration of antithymocyte globulin (ATG) to transplant recipients has been employed for some time. More recently, advances in monoclonal antibody technology have permitted the development of monoclonal antibodies specific for the CD3-T cell receptor complex to be used for this purpose. For example, Cosimi et al., *The New England Journal of Medicine*, Vol. 305, No. 6, pp. 308-314, August 6, 1981, and the Ortho Multi-Center Transplant Study Group, *The New England Journal of Medicine*, Vol. 313, No. 6, pp. 337-342, August 8, 1985, disclose the use of monoclonal antibody OKT3 in the treatment of renal transplant patients. OKT3 is a murine monoclonal antibody of IgG2a isotype specific for the CD3-T cell antigen receptor complex (see U.S. Patents Nos. 4,361,549, 4,515,893, and 4,654,210). Martin et al., *Transplantation Proceedings*, Vol. XVI, No. 6, pp. 1494-1495, 1984, and Remlinger et al., *Human Immunology*, Vol. 9, pp. 21-35 (1984) disclose the use of murine IgG2a monoclonal antibodies 9.6 (anti-CD2), 10.2 (anti-CD5), 12.1 (anti-CD6), 35.1 (anti-CD2), and 64.1 (anti-CD3) in the treatment of acute graft-versus-host disease. However, the *in vivo* use of anti-CD3 murine IgG2a monoclonal antibodies, including OKT3, has been associated with acute side effects, including pyrexia, chills, dyspnea, chest pain, vomiting, wheezing, nausea, diarrhea, tremor, and increased susceptibility to cytomegalovirus and herpes simplex infection. In addition, the use of murine IgG2a anti-CD3 monoclonal antibodies, specifically antibody 64.1, as an immunosuppressive agent for the treatment of acute GVHD has been documented to be associated with a substantial risk of EBV-associated lymphoproliferative disorders (see Martin et al., *Annals of Internal Medicine*, Vol. 101, pp. 310-315, 1984 and Martin et al., *American Journal of Kidney Diseases*, Vol. XI, No. 2, pp. 149-152, Feb. 1988. EBV-associated lymphoproliferative disorders have similarly been associated with administration of the OKT3 antibody.

The EBV-related lymphoproliferative syndrome has emerged as a major complication in attempts to treat graft rejection and acute GVHD with monoclonal antibodies. Despite the advances that have been made in

immunosuppression therapy with monoclonal antibodies, particularly with the IgG2a anti-CD3 monoclonal antibodies OKT3 and 64.1, a major need exists for improved monoclonal antibodies which are effective as immunosuppressive agents, but which are not associated with acute toxicity and do not enhance the risk of B-cell proliferation and transformation.

It is accordingly one object of this invention to provide hybridomas which produce monoclonal antibodies specific for the human CD3-T cell antigen receptor complex.

It is a further object of the invention to provide monoclonal antibodies specific for the CD3-T antigen receptor complex which are not associated with acute toxicity and do not increase the risk of EBV-associated lymphoproliferative syndrome when administered *in vivo*.

It is a still further object of the invention to provide methods and therapeutic compositions for the treatment or prophylaxis of transplant rejection and graft-versus-host disease.

Other objects and advantages of the invention will become apparent from an examination of the present disclosure.

Summary of the Invention

There have now been discovered novel hybridomas which are capable of producing monoclonal antibodies specific for the CD3 differentiation cluster of human T lymphocytes. The antibodies are effective as immunosuppressive agents when administered *in vivo*, but are not pyrogenic, do not induce respiratory distress, and do not increase the risk of EBV-related lymphoproliferative syndrome. Accordingly, the antibodies are effective in the treatment or prophylaxis of organ or bone marrow rejection by a transplant recipient, in the treatment or prophylaxis of graft-versus-host disease, or for diagnostics purposes.

Preferred antibodies of the invention are murine monoclonal antibodies of the IgG2b isotype which do not interact with Fc receptors on human monocytes. A representative embodiment of the invention is the hybridoma designated BC3 which produces a monoclonal antibody, also designated BC3.

The hybridoma BC3 has been deposited with the American Type Culture Collection, 12301 Park Lawn Drive, Rockville, Maryland 20852, and assigned the ATCC accession No. HB 10166.

Brief Description of the Drawings

FIGURE 1 is a graphic representation of the comodulation of the epitopes on peripheral blood lymphocytes bound by either antibody 64.1 or antibody BC3, demonstrated by staining with either FITC-conjugated antibody BC3 or FITC-conjugated antibody 64.1, respectively.

FIGURE 2 is a graphic representation of cell proliferation of peripheral blood mononuclear cells induced by antibody BC3 or antibody 64.1, as measured by thymidine incorporation assay.

FIGURE 3 is a graphic representation of cell proliferation of peripheral blood mononuclear cells induced by an alloantigen in the presence of antibody BC3, as measured by thymidine incorporation assay.

Detailed Description of the Invention

As used herein, the term "monoclonal antibody" means an antibody composition having a homogenous antibody population. It is not intended to be limited as to the source of the antibody or the manner in which it is made.

As used herein with respect to the exemplified murine monoclonal anti-CD3 antibodies, the term "functional equivalent" means a monoclonal antibody having selective binding affinity for the CD3-T antigen receptor complex of human T lymphocytes (T cells) and which does not bind to human monocyte Fc receptors.

As used herein with respect to the exemplified hybridomas of the invention, the term "progeny" is intended to include all derivatives, issue, and offspring of the parent hybridoma that produce the monoclonal antibody of the parent or a functional equivalent thereof, regardless of generation of karyotic identity.

Antibodies of the invention specifically bind the Tp19-29 antigen of the CD3 differentiation cluster of human T lymphocytes as defined in Bernard et al. (Eds.), *in Joint Report of the First International Workshop on Leukocyte Differentiation Antigens*, New York, pp. 9-142 (Springer-Verlag, 1984). Unlike well-known anti-CD3 monoclonal antibodies OKT3 and 64.1, however, the antibodies of the invention are of the IgG2b isotype and do not interact with Fc receptors on human monocytes (see Kenneth et al., *European Journal of Immunology*, Vol. 16, pp. 478-486, 1986). Accordingly, the *in vivo* administration of the monoclonal antibodies of the invention does not result in the inducement of EBV-related lymphoproliferative syndrome, febrile reactions, bronchospasm or hypotension typically present after administration of the IgG2a anti-CD3 monoclonal antibodies OKT3 and 64.1, complications which are believed to result from the secretion of lymphokines after antibody binding to T cells crosslinks to Fc receptor position accessory cells.

A presently particularly preferred, representative embodiment of the antibodies of the invention is the antibody BC3 produced by the BC3 hybridoma (ATCC No. HB 10166) and functional equivalents thereof.

Antibody-producing lymphocytes used as fusion partners to make the hybridomas of the invention may be generated by immunizing mice, such as

BALB/c mice, with PHA activated T cells or membrane extracts made therefrom. The mice are preferably innoculated intraperitoneally with an immunogenic amount of the cells or extract and then preferably boosted with similar amounts of the immunogen over a period of about 10 days to two weeks. Spleens may be collected from the immunized mice after the final booster injection and processed to obtain a cell suspension for use in fusion with myeloma cells.

Myeloma cells used as fusion partners to make the hybridomas of the invention are well known and readily available in the art. In a representative embodiment, more fully described herein, BALB/c MOPC21 NS1/1 is used as the myeloma fusion partner, although other myeloma cell lines may be used for this purpose.

In producing the hybridomas of the invention, the spleen cells from immunized mice are combined with the myeloma cell fusion partners in the presence of a suitable fusion agent, such as polyethylene glycol. After fusion, the cells are separated from the fusion medium and grown in a selective medium, such as HAT medium, to eliminate nonhybridized parent cells. Supernates from the remaining hybridomas are screened for specific binding affinity for the CD3-T cell antigen receptor complex of human peripheral T cells and lack of binding affinity for human monocytes by conventional assay procedures, such as by radioimmunoassay, enzyme immunoassay, fluorescence immunoassay, or the like. In a representative embodiment, hybridomas of interest may be selected by screening supernates for blocking the binding of a fluorescence in isothiocyanate (FITC) conjugated anti-CD3 antibody by using flow microfluorimetry. Specifically, 0.5×10^6 peripheral blood mononuclear cells obtained from volunteers and purified by density gradient centrifugation on Ficoll Hypaque (S.g. 1077) may be incubated with 50µl of hybridoma culture supernates for 30 min. at 4°C in the presence of 0.1% sodium azide. Cells may then be incubated with saturating concentration of a known CD3-specific antibody conjugated to FITC. Incubation may be carried out for 30 min. at 4°C in the presence of 0.1% sodium azide. After washing, cells may be evaluated by flow microfluorimetry. Green fluorescence intensity of samples preincubated with hybridoma supernates may be compared to green fluorescence intensity of samples preincubated with medium. Hybridoma supernates capable of reducing the fluorescence intensity of cells stained with FITC-conjugated CD3 antibody should contain an antibody specific for the CD3 antigen.

Supernates found to contain a CD3 antibody may be further screened for the presence of antibodies of the desired IgG2b isotype as follows. 50 µl of hybridoma supernate containing the newly found anti-CD3 antibody may be incubated with 0.5×10^6 unfractionated peripheral blood mononuclear cells in 200 µl round bottom well plates for 72 hours at 37°C in the presence of 5% CO_2 atmosphere. Induction of DNA synthesis may be measured by incorporation of tritiated thymidine. Since antibodies of IgG2a, IgG1 and IgG3 isotype are known to be mitogenic for unfractionated peripheral blood mononuclear cells, supernates containing antibodies inducing DNA synthesis are discarded. Supernates containing anti-CD3 antibodies not inducing DNA synthesis may contain antibodies of the IgG2b or the IgM isotype. Anti-CD3 antibodies of the IgG2b isotype may then be identified by various conventional means known in the art, such as by ELISA (enzyme-linked immunosorbent assay) or double immunodiffusion, using antibodies or antisera specific for IgG2b antibodies.

Hybridomas determined by a suitable screening procedure to produce antibodies of the invention may be grown *in vitro* in culture medium or *in vivo*, preferably in the peritoneal cavity of a mouse, using conventional procedures. Monoclonal antibodies of the invention may be separated from the culture medium, or body fluids, such as ascites fluid or serum, and purified by known immunoglobulin purification procedures, such as ammonium sulfate precipitation, gel electrophoresis, dialysis, chromatography and ultrafiltration.

The foregoing and other aspects of the invention may be better understood in connection with the following examples, which illustrate a presently preferred representative embodiment of the invention.

EXAMPLE 1 - Hybridoma Production

Hybridoma BC3 was prepared by fusion of immune murine spleen cells with a murine myeloma cell line, substantially as described by Kohler and Milstein, *Nature*, Vol. 256, p. 495 *et seq.*, 1975. Specifically, a BALB/c mouse was immunized intraperitoneally with 2×10^6 to 2×10^7 1:1 cell mixture of PHA activated T cells on day 18, day 11 and day 4, before infusion. Spleen cells of the mouse were fused with $HGPRT^-$ myeloma cell line BALB/c MOPC21 NS1/1 obtained from Dr. Caesar Milstein (Molecular Research Council, Cambridge, England). Selected hybrid cells were subcultured at low density, together with a "feeder cell" suspension of thymocytes from BALB/c mice. These cell mixtures contained hybrid cells at a concentration of 2.5×10^1/ml and thymocytes at a concentration of 4×10^6/m9. A 200 µl volume of this suspension was dispensed into microwells, resulting in a seeding of 5 hybrid cells/well. Hybridoma culture

supernates were screened, as described above, for anti-CD3 specificity and IgG2b isotype, and cloning of cells from a positive well was performed by limiting dilution (100 hybridoma cells/300 wells) with thymocyte feeder cells as described above, to obtain BC3 hybridoma cells. The hybridoma cells were cultured in RPMI 1640 containing 15% fetal calf serum, 2 mM added glutamine, 1 mM pyruvate, 100 units/ml penicillin, and 1 mg/ml streptomycin. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2. Cell density was maintained between 0.1×10^6/ml and 0.4×10^6/ml by splitting the cultures every two to three days.

EXAMPLE 2 - BC3 Ascites Fluid Production

BALB/c mice were pretreated with 0.5 ml of intraperitoneal pristane (Alderidge, Milwaukee, WI), and then innoculated by intraperitoneal injection of 5-10×10^6 BC3 hybridoma cells prepared in accordance with Example 1. After five to 15 days, ascites fluid was obtained by insertion of an 18 gauge needle into the peritoneum and collection by open draining into a sterile 5 ml tube. Fluids were allowed to clot at room temperature for one-half hour, and then centrifuged at 2,500 x G for 10 minutes at 4°C. Multiple ampules of hybridoma cells from ascites fluid of individual selected mice were cryo-preserved in 50% RPMI 1640 medium and 10% DMSO.

EXAMPLE 3 - Antibody Purification

Ascites fluid obtained according to Example 2 was purified by sequential G-25 Sephadex filtration and affinity chromatography on Protein A-Sepharose CL-4B to obtain purified BC3 monoclonal antibody. All handling of monoclonal antibodies was done with disposable pharmaceutical syringes or other pyrogen-free instruments. Protein A-Sepharose CL-4B columns were stored at 4°C in phosphate-buffered saline (PBS) pH 7.2 containing 0.1% Na azide. Columns were purged with 3 bed volumes of PBS before application of ascites fluid. Where possible, pharmaceutical-grade reagents were employed in preparation of buffers.

EXAMPLE 4

Antibody Characterization and Class Determination

Supernate from the BC3 hybridoma of Example 1 contained a murine IgG2b/k antibody, designated BC3, as determined by an ELISA assay using peroxidase-conjugated rabbit anti-mouse Ig isotype specific antibodies.

The immunoprecipitate of ^{125}I surface labeled human PBL lysate with antibody BC3 consisted of a protein complex with a molecular weight of 19-29 kDa by one-dimensional SDS-PAGE. The specificity of BC3 for the CD3 complex was demonstrated by comodulation experiments with the known CD3 antibody

64.1. In the comodulation experiments, 5×10^5 peripheral lymphocytes were incubated for 16 hours at 37°C with either medium alone or with antibody BC3 and then stained at 4°C for 30 min with FITC-conjugated antibody 64.1, or with antibody 64.1 and stained FITC-conjugated BC3. The fluorescence intensity of antibody bound to the lymphocytes was then determined. As shown in FIG. 1, the fluorescence intensity associated with lymphocyte-bound FITC-conjugated antibody BC3 (represented by the medium alone) is substantially reduced in the presence of antibody 64.1; and, conversely, the fluorescence intensity associated with lymphocyte-bound FITC-conjugated antibody 64.1 (represented by the medium alone) is substantially reduced in the presence of antibody BC3. This experiment demonstrated that both the BC3 and 64.1 antibodies bind to the same CD3 molecular complex.

The BC3 and 64.1 antibodies were further evaluated to characterize their mitogenic properties. Five x 10^4 peripheral blood mononuclear cells were incubated at 37°C in a 5% CO_2 atmosphere in the presence of 10µg of antibody 64.1, antibody BC3 or antibody 9E8 (an irrelevant antibody control). At various time intervals, cell proliferation was measured by thymidine incorporation assay, as described in Schooley et al., "T-lymphocyte subset interactions in the cell-mediated immune response to Epstein-Barr virus." *Cell Immunol.*, Vol. 86, pp. 402-412, 1984. As shown in FIG. 2, antibody 64.1 induced cell proliferation, which was maximal on days 3 and 4, whereas antibody BC3 did not induce any cell proliferation.

The effect of antibody BC3 on T cell proliferative response was further characterized as follows. Five x 10^4 peripheral blood mononuclear cells were incubated with an equal number of HLA-class II, incompatible, irradiated (3000 cGy) peripheral blood mononuclear cells in the presence of 10 µg/ml of antibody BC3 or 9E8 (an irrelevant antibody control). At various time intervals, cell proliferation was measured by thymidine incorporation assay. As shown in FIG. 3, antibody BC3 completely abrogated cellular proliferative response to alloantigens.

EXAMPLE 5

Immunosuppressive Activity and Effect on Lymphoproliferation

To evaluate whether BC3 antibody has immunosuppressive properties without the toxicities associated with administration of the other CD3 antibodies, a clinical study was undertaken using escalating doses of BC3 for treatment of patients with acute GVHD refractory to corticosteroids. Ten patients were treated with the antibody. One of 65 infusions was followed by fever and none by other acute reactions. One patient who had been previously treated with ATG

subsequently received seven infusions of antibody BC3 for treatment of skin GVHD. After an initial response to BC3 treatment, a second course of ATG was administered for treatment of recurrent disease. Later, this patient developed a lethal EBV-LPS. Because of the confounding role of ATG treatment and the other risk factors, namely an HLA-incompatible transplant, immunosuppressive treatment with cyclosporine and corticosteroids and severe acute GVHD, the contributory role played by antibody BC3 in the development of EBV-LPS could not be reliably assessed. None of the other patients developed this complication.

Three patients were not evaluable for clinical response: two did not live long enough to complete the treatment course, and one had liver GVHD superimposed on pretransplant hepatitis and veno occlusive disease. Seven patients were evaluated for response: disease resolved in all seven cases, skin in four, liver in one, and both skin and liver in two. A BC3 antibody dose of 0.1/mg/kg/day was found to be effective in all patients except two, who had the dose doubled after three days because of persistent GVHD. In one case, the response was not sustained and the patient required subsequent treatment with ATG, as described above. In the other six cases, the response was sustained until death from veno occlusive disease of the liver (one), and CMV pneumonia (two), or indefinitely in the three patients surviving more than 100 days after transplantation. From these data, it is concluded that administration of antibody BC3 is not associated with acute toxicity and that it is effective treatment of GVHD.

When used *in vivo* for therapy, the immunosuppressive antibodies of the invention are administered to a patient in therapeutically or prophylactically effective amounts, i.e., amounts sufficient to eliminate or reduce T-cell interaction with transplanted organ or bone marrow tissue in a transplant recipient and thereby reduce or eliminate rejection of the organ or bone marrow transplant. The antibodies will typically be administered parenterally, preferably intravenously, but may be administered by other routes known in the art. Suitable dose and dosage regimens will depend upon the desires of the treating physician, the nature, history and medical condition of the patient, and the specific condition being prophylactically or therapeutically treated. Typical therapeutically or prophylactically effective amounts of antibody for intravenous administration will range from about 0.05 mg/kg/day to about 10 mg/kg/day of patient weight for periods of from about two to about 14 days, or longer.

For parenteral administration, antibodies of the invention will be formulated in unit dosages in injectable form in association with a pharmaceutically

acceptable parenteral carrier. Suitable, acceptable carriers are typically nontoxic and nontherapeutic. Examples of suitable carriers include saline, Ringer's solution, and human serum albumin solutions. Nonaqueous vehicles, such as fixed oils and ethyloleate and liposomes may also be employed. Suitable vehicles may additionally contain minor amounts of additives, such as substances that enhance isotonacity and chemical stability, e.g., buffers and preservatives. Antibody will typically be present in the pharmaceutical carrier for direct administration at final concentrations of from about 0.05 mg/ml to about 5.0 mg/ml.

Antibodies of the invention may be administered alone, or in conjunction with other immunosuppressive agents, if desired. Other representative immunosuppressive agents suitable for use in conjunction with the antibodies of the invention include the immunosuppressive monoclonal antibodies, corticosteroids, azathioprine, methotrexate, cyclosporine and antithymocyte globulin.

While the invention has been described in connection with certain presently preferred, illustrative embodiments, it is apparent that various modifications, such as use of the antibodies of the invention for diagnostic or other purposes, can be made by one skilled in the art without departing from the spirit and scope of the invention. Except as precluded by the prior art, any such modifications are intended to be within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A hybridoma which produces murine monoclonal antibodies reactive with the CD3-T cell antigen receptor complex of human peripheral T cells and not reactive with the Fc receptors of human monocytes.

2. A hybridoma of Claim 1, which produces IgG2b isotype antibodies.

3. A hybridoma of Claim 2 having the characteristics of hybridoma BC3 (ATCC No. HB 10166).

4. A monoclonal antibody which reacts with the CD3-T cell antigen receptor complex of normal human peripheral T cells, but does not react with the Fc receptors of human monocytes.

5. A monoclonal antibody of Claim 4 having an IgG2b isotype.

6. A monoclonal antibody of Claim 5 having the characteristics of that produced by hybridoma BC3 (ATCC No. HB 10166).

7. A method of producing a murine monoclonal antibody reactive with the CD3-T cell antigen receptor complex of human peripheral T cells, but not reactive with the Fc receptors of human monocytes, comprising culturing the hybridoma BC3 (ATCC No. HB 10166) in a suitable medium and recovering antibody produced by the hybridoma.

8. Monoclonal antibodies produced by the method of Claim 7.

9. A method of producing murine monoclonal antibody reactive with the CD3-T cell antigen receptor complex of human peripheral T cells, but not reactive with human monocytes comprising injecting the hybridoma BC3 (ATCC No. HB 10166) into the peritoneal cavity of a mouse and recovering antibody from ascites fluid or serum of the mouse.

10. Monoclonal antibodies produced by the method of Claim 9.

11. A therapeutic composition comprising an amount of a murine monoclonal antibody reactive with the CD3-T cell antigen receptor complex of human peripheral T cells and not reactive with human monocytes, effective to reduce or eliminate rejection of an organ or bone marrow transplant by a transplant recipient, or effective to reduce or eliminate graft-versus-host disease (GVHD), together with a pharmaceutically acceptable carrier.

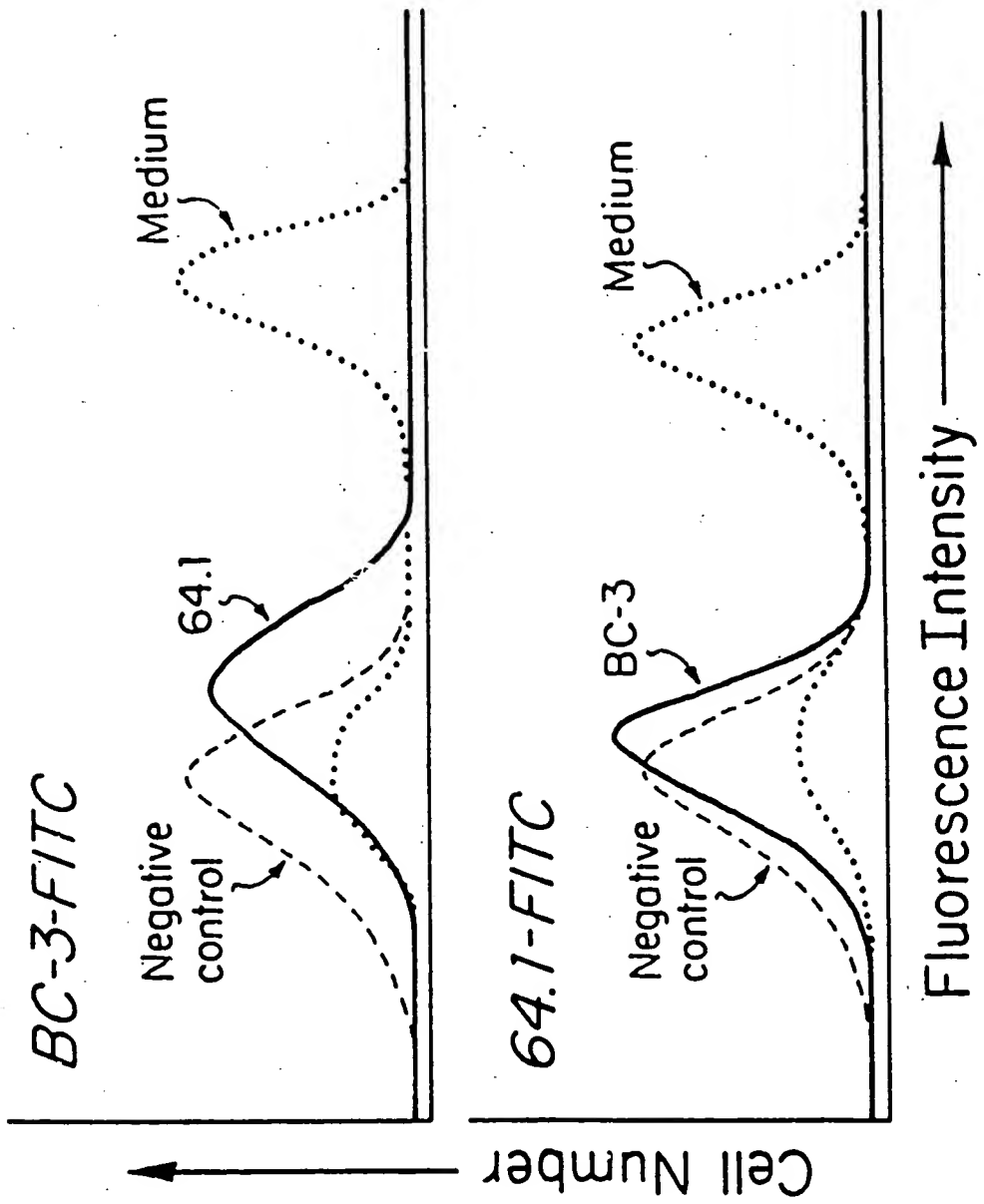
12. A therapeutic composition comprising an amount of a murine monoclonal antibody produced from a hybridoma having the characteristics of BC3 (ATCC No. HB 10166), effective to reduce or eliminate rejection of an organ or a bone marrow transplant by a transplant recipient, or effective to reduce or eliminate graft-versus-host disease (GVHD), together with a pharmaceutically acceptable carrier.

13. A method of treatment of an organ or bone marrow transplant recipient comprising administering to the recipient an amount of a murine monoclonal antibody reactive with the CD3-T cell antigen receptor complex of human peripheral T cells and not reactive with human monocytes, effective to cause reduction or elimination of rejection of the transplant by the recipient.

14. The method of Claim 13, wherein the monoclonal antibody is produced from a hybridoma having the characteristics of BC3 (ATCC No. HB 10166).

15. A method of treatment of a transplant patient comprising administering to the patient an amount of a murine monoclonal antibody reactive with the CD3-T-cell antigen receptor complex and not reactive with the Fc receptors of human monocytes, effective to reduce or eliminate graft-versus-host disease (GVHD).

16. The method of Claim 15, wherein the monoclonal antibody is produced from a hybridoma having the characteristics of BC3 (ATCC No. HB 10166).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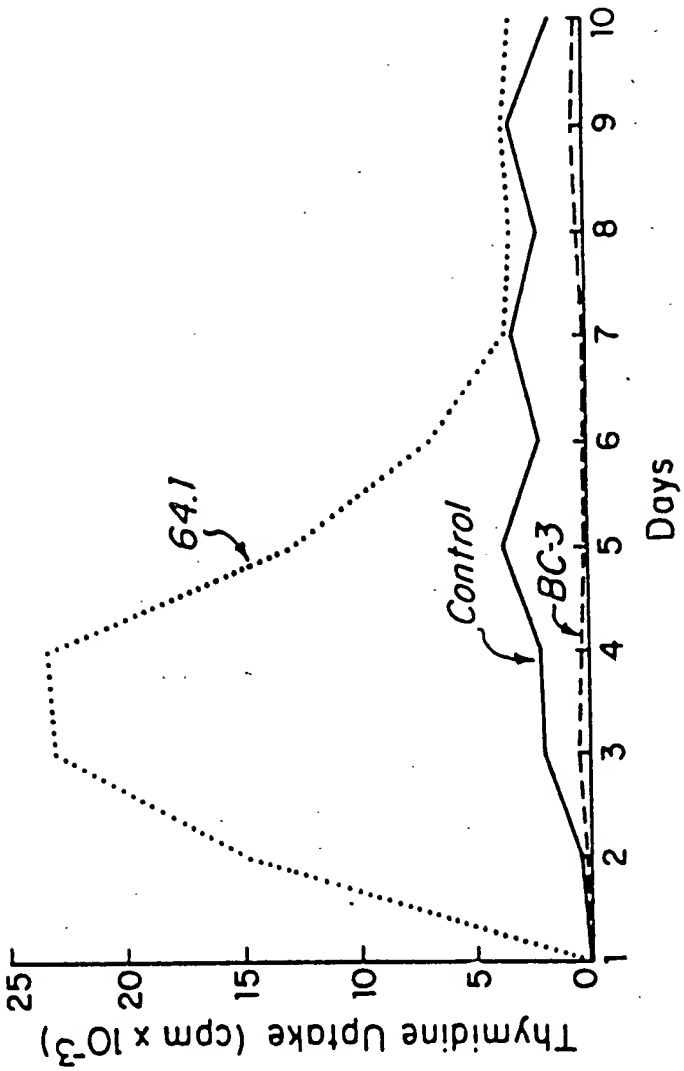
64.1
Negative control
64.1-FITC
Medium
BC-3
Negative control
Cell Number
Fluorescence Intensity

Fig. 1.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15
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6
7
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9
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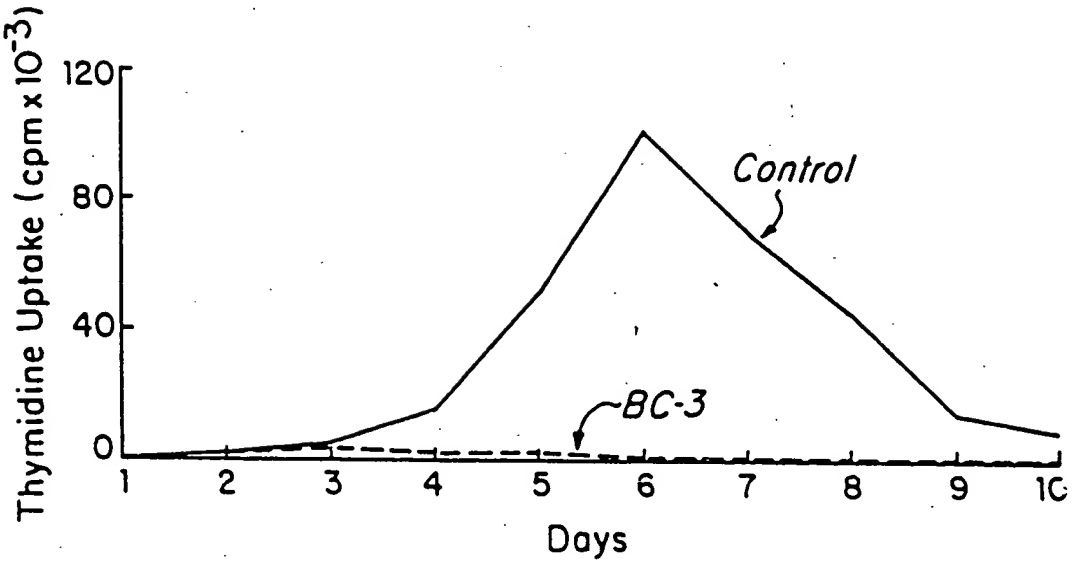
Days
64.1
Control
BC-3

Fig. 2.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0
1
2
3
4
5
6
7
8
9
10
Days
Control
BC-3

Fig. 3.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/04215

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 39/395
US.CL.: 424/85.8

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	424/85.8 435/240.27,70.21 530/387

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [6]

Databases: Dialog (Files 5,10,155,399,238,72,173), USPTO Automated Patent System (File USPAT, 1971-1990).

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
Y	Scand. J. Immunol., Volume 28, issued 1988, Preijers, F.W.M.B. et al., "Human T Lymphocyte Differentiation Antigens As Target For Immunotoxins Or Complement-Mediated Cytotoxicity", pages 185-194, see the entire document.	11-13 and 15
Y	Transplantation, Volume 44, No. 1, issued 1987, Filipovich et al., "Graft-Versus-Host Disease Prevention in Allogeneic Bone Marrow Transplantation From Histocompatible Siblings", pages 62-69, see the entire document.	11-13 and 15
Y	A.M. Campbell, "Laboratory Techniques in Biochemistry and Molecular Biology: Monoclonal Antibody Technology" published 1984 by Elsevier, see especially chapters 1, 3, 4, 5, 6 and 8.	7-16

* Special categories of cited documents: [15]

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
09 October 1990	08 JAN 1991
International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	Susan L. Futrovsky

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	A.J. McMichael, "Leucocyte Typing III: White Cell Differentiation Antigens" published 1987 by Oxford University Press, see especially pages 932-937.	1 and 4

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . . . , because they relate to subject matter [1] not required to be searched by this Authority, namely:

2. ☐ Claim numbers . . . , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [1], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.